

DATA EVALUATION RECORD
WHOLE SEDIMENT ACUTE TOXICITY INVERTEBRATES, FRESHWATER
OPPTS Guideline 850.1735

1. **CHEMICAL:** Cypermethrin PC Code No.: 109702
2. **TEST MATERIAL:** Cypermethrin Technical 40/60 Purity: 40.6% cis/59.4% trans

3. **CITATION:**

Authors: Picard, C.R.
Title: 10-Day Toxicity Test Exposing Freshwater Amphipods
(*Hyalella azteca*) to Cypermethrin Applied to California
Sediment 3 Under Static-Renewal Conditions.

Study Completion Date: May 15, 2009

Laboratory: Springborn Smithers Laboratories
790 Main Street
Wareham, MA 02571

Sponsor: Pyrethroid Working Group
Beverage & Diamond
1350 I Street NW
Washington, DC 20005

Laboratory Report ID: 13656.6128
MRID No.: 47946605
DP Barcode: 420006

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: 

Date: 06/10/10

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

Signature: 

Date: 06/11/10

5. **APPROVED BY:** Stephen Carey, Biologist, OCSPP/EFED/ERB6

Signature: 

Date: 7/21/15

6. **STUDY PARAMETERS:**

Age of Test Organism:	7 to 8 days old
Definitive Test Duration:	10 days
Study Method:	Intermittent flow-through
Type of Concentrations:	Mean-measured

7. CONCLUSIONS:

Results Synopsis:

Based upon mean-measured sediment concentrations:

Survival:

LC₅₀: 60 µg a.i./kg 95% C.I.: 53 to 68 µg a.i./kg
Slope: N/A
NOAEC: 63 µg a.i./kg
LOAEC: 90 µg a.i./kg

Growth:

EC₅₀: 53 µg a.i./kg 95% C.I.: 42 to 65 µg a.i./kg
Slope: 3.54±0.78
NOAEC: 24 µg a.i./kg
LOAEC: 63 µg a.i./kg

Based upon ESTIMATED¹ pore water concentrations:

Survival:

LC₅₀: 0.007 µg a.i./L 95% C.I.: 0.006 to 0.007 µg a.i./L
NOAEC: 0.007 µg a.i./L
LOAEC: 0.01 µg a.i./L

Growth (dry weight):

IC₅₀: 0.006 µg a.i./L 95% C.I.: 0.005 to 0.007 µg a.i./L
Slope: 3.54±0.78
NOAEC: 0.003 µg a.i./L
LOAEC: 0.007 µg a.i./L

Based upon OC-normalized mean-measured sediment concentrations:

Survival:

LC₅₀: 938 µg a.i./kg TOC 95% C.I.: 828 to 1062 µg a.i./kg TOC
Slope: N/A
NOAEC: 984 µg a.i./kg TOC
LOAEC: 1406 µg a.i./kg TOC

1 Freely dissolved pore water endpoints (ug/L) estimated as:

Mean measured bulk sediment conc. (ug/kg-dw) / [Fraction TOC (kg OC/kg-dw) * K_{OC} (L/kg-OC)]

Growth (dry weight):

EC₅₀: 828 µg a.i./kg TOC

Slope: 3.54±0.78

NOAEC: 375 µg a.i./kg TOC

LOAEC: 984 µg a.i./kg TOC

95% C.I.: 656 to 1016 µg a.i./kg TOC

8. ADEQUACY OF THE STUDY:

A. Classification: ACCEPTABLE

B. Rationale: N/A

C. Repairability: N/A

9. MAJOR GUIDELINE DEVIATIONS:

No major deviations noted.

10. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: <i>H. azteca</i> or <i>Chironomus tentans</i>	<i>Hyaella azteca</i>
Life Stage: For <i>C. tentans</i> : third instar (9-11 days old). The instar stage of midges must be confirmed by head capsule width (approx. 0.38 mm). For <i>H. azteca</i> : 7- to 14-day old amphipods must be produced. If growth is also an endpoint, a narrower range, such as 1- to 2-day old amphipods should be used.	7 to 8 days old
Supplier Brood stock can be obtained from laboratory, commercial, or government sources. (Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.)	Amphipods originated from laboratory cultures maintained in <i>ca.</i> 15 L of culture water (same source as dilution water) under flow-through conditions.

Guideline Criteria	Reported Information
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period: The required culture and testing temperature is 23°C. The test organisms should be cultured in the same water to be used for testing.	Adults were removed from the main culture tanks 7 to 10 days prior to test initiation and placed in <i>ca.</i> 8 L of water. Juvenile amphipods (<24 hours old) produced by the isolated adults were then transferred to <i>ca.</i> 0.80 L of laboratory dilution water and reared under static conditions for 7 to 8 days with gentle aeration. During the holding period, the dissolved oxygen ranged from 7.3 to 8.3 mg/L and temperature ranged from 23 to 24 °C.
Feeding:	During holding and acclimation, amphipods were fed every other day with 2.5 mL of a combination of yeast, cereal leaves, and flaked fish food suspension (YCT) and 2.5 mL of <i>Ankistrodesmus falcatus</i> .
Pretest Mortality: A group of organisms should not be used if they appear unhealthy, discolored (eg <20% mortality 48 h before the beginning of a test).	No mortality during the 48 hours prior to test initiation.

C. Test System

Guideline Criteria	Reported Information
<p>Source of dilution water (overlying water) and sediment: Soft reconstituted water or water from a natural source. Tap water is acceptable if it is dechlorinated, deionized, and carbon filtered, but its use is not encouraged.</p> <p>Uncontaminated natural sediment is recommended.</p>	<p>Laboratory well water characterized as having a total hardness and total alkalinity as CaCO_3 of 42 to 44 and 20 mg/L, respectively, a pH range of 6.8 to 7.0, and a specific conductance range of 220 to 230 $\mu\text{mhos/cm}$. Monthly analysis of the water source indicated a TOC 0.41 mg/L for March 2009.</p> <p>Natural freshwater sediment (Batch No. EMP0037) collected on November 21, 2008 from the Sacramento-San Joaquin Delta, CA and designated as CA Sediment 3. The sediment was wet-pressed sieved (2.0-mm) prior to use.</p>
Does water support test animals without observable signs of stress?	Yes.
<p>Quality Of Water If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 $\mu\text{g/L}$</p>	<p>There were no apparent problems with water quality.</p> <p>During the study, ammonia levels (as N) in the overlying water were ≤ 1.6 mg/L.</p>
<p>Water Temperature 23°C for both species. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1°C and 3°C, respectively.</p>	<p>Daily: 22 to 24°C Continuous: 22 to 25°C</p>
<p>pH Should not vary more than 50%. Survival is best at pH >6.5 for <i>C. tentans</i>.</p>	6.9 to 7.3
<p>Dissolved Oxygen Maintained between 40 and 100%.</p>	<p>4.8 to 8.0 mg/L (55 to 95% ASV at 22 to 24°C)</p>

Guideline Criteria	Reported Information
Total Hardness Should not vary more than 50%. <i>H. azteca</i> are sensitive to hardness (e.g., they are not found in waters with calcium at <7 mg/L and DO at <2 mg/L).	52 to 60 mg/L as CaCO ₃
Conductivity Should not vary more than 50%.	290 to 330 µmhos/cm
Sediment Characterization All sediment must be characterized for: pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content.	Particle distribution – 5% sand, 44% silt, 51% clay (silty clay; reviewer-derived from USDA soil texture triangle) Organic carbon content – 6.4% Solids – 19.65% pH – 5.8 Ammonia concentration of pore water – not reported
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	None reported
Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.	<u>Cypermethrin Technical 40/60</u> Synonym: FMC 30980 IUPAC Name: (RS)-α-cyano-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate CAS Name: cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate CAS No.: 52315-07-8 Description: not reported Lot No.: PL07-0633 Purity: 40.6% cis-isomer, 59.4% trans-isomer Storage: dark, room temperature

Guideline Criteria	Reported Information
<p>Stock Solutions Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.</p>	<p>Two hundred fifty (250) mL of a 20-µg a.i./mL stock solution was prepared in acetone. From this, five individual dosing solutions were prepared by diluting the appropriate amount of stock solution with acetone to 10 mL.</p> <p>All dosing solutions were clear and colorless, with no visible un-dissolved test substance.</p> <p>Negative and solvent controls were included in the test.</p>
<p>Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.</p>	<p>A jar-rolling technique was used to apply the test substance to the sediment. A 9-mL volume of the appropriate prepared dosing stock solution (in acetone) was applied to 0.050 kg of fine silica sand in glass Petri dishes, and the solvent was allowed to evaporate off for 60 minutes. The dry sand was then added to 4.0 kg of wet sediment (total of 0.8360 kg dw) in individual 1-gallon jars. Each jar was then rolled for 4 hours at room temperature at approx. 15 rpm. The jars were stored upright at 4 ± 2 °C during conditioning.</p> <p>The treated sediments were allowed to equilibrate for a 14-day period in the refrigerator. Twice a week during the conditioning period and prior to addition to the exposure vessels (day -1), the jars were mixed on the rolling mill for an additional 2 hours at room temperature to ensure the sediment was homogeneous.</p> <p>The range of concentrations (1.9 to 30 µg a.i./kg) was based upon the results of a preliminary range finding study.</p>

Guideline Criteria	Reported Information
Test Aquaria 1. <u>Material</u> : Glass or stainless steel or perfluorocarbon plastics. 2. <u>Size</u> : 300 ml high-form lipless beakers containing 100 ml of sediment and 175 ml of overlying water.	300-mL glass vessels containing 100 mL (approx. 4.0-cm layer) of sediment (equivalent to 21 g dw) and 175 mL of overlying water. The total overlying water plus sediment volume was maintained at <i>ca.</i> 275 mL. Test vessels were covered with 40-mesh Nitex® screen for drainage.
Type of Dilution System Daily renewal or a flow-through system may be used.	Intermittent flow-through
Flow Rate 2 volume changes/day	2 volume additions/day
Aeration Dilution water should be vigorously aerated prior to use so that dissolved oxygen in the overlying water remains above 40% saturation.	None reported
Photoperiod 16 hours light, 8 hours dark at 500 to 1000 lux.	16 hours light, 8 hours dark; 880 to 1000 lux
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used, it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	Acetone, 9 mL per 0.8360kg dw sediment. The acetone was allowed to completely evaporate during the mixing procedure.

D. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment.	One day prior to the addition of amphipods (day -1), the test systems were established. Overlying water was gently added, and each vessel was placed under the renewal system.
Renewal of Overlying Water: Renewal of the overlying water should be conducted on day -1 prior to the addition of organisms or food on day 0. For flow-through systems, the flow rates should not vary by more than 10% between any two chambers at any time. Proper operation should be verified by calibration prior to test initiation.	The overlying water was replaced twice daily using an intermittent delivery system in combination with a calibrated water-distribution system. The test system was calibrated before and after the test, and visually inspected at least twice daily for proper functioning.
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	Amphipods were impartially assigned one or two at a time into intermediate test beakers until all beakers contained ten amphipods. The test was initiated when each intermediate beaker of amphipods was added to each respective test vessel.

Guideline Criteria	Reported Information
<p>Range Finding Test A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment.</p>	<p><u>Preliminary toxicity assessment</u></p> <ul style="list-style-type: none"> • Treated sediment equilibrated for 7 days • 10-day exposure at nominal levels of 0 (negative and solvent controls), 0.020, 0.20, 2.0, 20, and 200 µg a.i./kg • three replicates per level, each containing 10 organisms • Survival averaged 87 (negative control), 87 (solvent control), 90, 97, 80, 80, and 0% respectively • Dry weight averaged 0.13 (negative control), 0.09 (solvent control), 0.08, 0.09, 0.07, and 0.07 mg, respectively
<p>Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.</p>	<p>Test vessels were observed daily for mortality and abnormal behavior.</p>
<p>Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.</p>	<p>0 (negative and solvent controls), 13, 25, 50, 100, and 200 µg a.i./kg sediment</p>
<p>Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.</p>	<p>80 amphipods per level, with 10 amphipods per replicate vessel and 8 biological replicates per level</p> <p>An additional 20 replicates were maintained for chemical analysis</p>
<p>Test organisms randomly or impartially assigned to test vessels?</p>	<p>Yes</p>

Guideline Criteria	Reported Information
<p>Feeding <i>C. tentans</i> in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin⁷ suspension daily. <i>H. azteca</i> may be fed with a mixture of yeast, Cerophyl, and trout chow (YCT) at a rate of 1.5 mL daily per test chamber. A drop in DO levels below 2.5 mg/L may indicate over-feeding and feeding should be suspended in all treatments until DO levels increase.</p>	<p>1.0 mL of yeast, cereal leaves, and flaked fish food suspension (YCT) once daily.</p>
<p>Water Parameter Measurements Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of the test.</p> <p>DO should be measured daily.</p> <p>Temperature should be measured daily in one test chamber from each treatment. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1 and 3°C, respectively.</p>	<p><u>Overlying water:</u> For all levels, total hardness, alkalinity, specific conductance, and ammonia concentrations were measured in a composite sample on Days 0 and 10.</p> <p>DO, temperature, and pH were measured in each replicate vessel on Days 0 and 10 and in one alternating replicate from each level on Days 1 to 9. Temperature was also continuously monitored in an auxiliary vessel in the water bath.</p> <p><u>Pore water:</u> Redox potential, pH, ammonia, and dissolved organic carbon (DOC) were measured in a composite sample on Days 0 and 10.</p>

Guideline Criteria	Reported Information
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	<p>Surrogate samples vessels were collected on Days 0 and 10, and concentrations of cypermethrin were determined in pore water and sediment (see Reviewer's Comments section). The sediment/pore water matrices were isolated by centrifuging for 15 to 30 minutes at 1200 g.</p> <p>Aliquots of the dosing stock solutions were analyzed for cypermethrin. In addition, treated sediment from all levels was analyzed for cypermethrin prior to the allocation of the sediment into the replicate vessels (following equilibration).</p>

11. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in accordance with GLP Standards as specified in 40 CFR 160 with the following exceptions: the routine water, sediment, and food contaminant screening analyses.
Control Criteria Was control mortality $\leq 20\%$? Were control <i>C. tentans</i> an average size of ≥ 0.6 g?	Mortality: Negative control – 10% Solvent control – 4%

Guideline Criteria	Reported Information
Percent Recovery of Chemical:	Procedural recoveries (QC samples) conducted concurrently with sample analysis: <u>Sediment:</u> 90.8 to 104% of nominal (excludes one outlier of 139%) <u>Aqueous:</u> 76.1 to 96.7% of nominal
Data Endpoints - Survival - Dry weight (determined by pooling all living organisms from a replicate and drying at 60 to 90°C to a constant weight) - Body length (amphipod only)	- Survival - Dry weight
Raw data included?	Yes, sufficient

Effects Data

Toxicant Concentration				Survival		Dry Weight	
Nominal (µg a.i./kg)	Mean Measured ^(a)						
	Sediment (µg a.i./kg dw)	Pore Water (µg a.i./L)	Overlying Water (µg a.i./L)	Mean %	% Inhibition	mg per larvae	% inhibition
Control	<LOQ	<LOQ	Not assessed	90	N/A	0.15	N/A
S. Control	<LOQ	<LOQ	Not assessed	96	-6.7	0.15	0
13	7.7	0.056	Not assessed	95	-5.6	0.14	6.7
25	13	0.024	Not assessed	100	-11	0.15	0
50	24	0.072	Not assessed	91	-1.1	0.14	6.7
100	63	0.11	Not assessed	61	32	0.05*	67
200	90	0.24	Not assessed	19*	79	0.03 ^(b)	80

^(a) LOQ were equivalent to 0.94 to 1.1 µg a.i./kg for sediment samples and 0.00085 to 0.00096 µg a.i./L for pore water samples.

^(b) Excluded from statistical analyses due to significant effect on survival.

* Statistically different ($p \leq 0.05$) compared to the negative control.

Other Significant Results:

Biological: After 10 days, survival averaged 90 and 96% for the negative and solvent controls, respectively, and 95, 100, 91, 61, and 19% for the mean-measured 7.7, 13, 24, 63, and 90 µg a.i./kg sediment levels, respectively. The data were non-homogenous (see statistical results sections), and the difference at the 90 µg a.i./kg sediment level was statistically-reduced ($p \leq 0.05$) compared to the negative control. The 10-day LC_{50} (with 95% C.I.) was reported to be 72 (57 to 78) µg a.i./kg sediment, and the NOAEC for survival was 63 µg a.i./kg.

After 10 days, dry weight averaged 0.15 mg per larvae at both of the negative and solvent control levels, and 0.14, 0.15, 0.14, 0.05, and 0.03 mg per larvae at the mean-measured 7.7, 13, 24, 63, and 90 µg a.i./kg sediment levels, respectively. The difference at the 63 µg a.i./kg sediment level was statistically-reduced ($p \leq 0.05$) compared to the negative control (the 90 µg a.i./kg level was not statistically compared due to the significant effect on survival at this level). The 10-day EC_{50} (with 95% C.I.) was reportedly 52 (47 to 58) µg a.i./kg sediment, and the NOAEC for amphipod growth was 24 µg a.i./kg.

Analytical: Concentrations of cypermethrin were determined on Days 0 and 10 in sediment and pore water only (see Reviewer's Comments section). In sediment, concentrations decreased 25 to 71% from Days 0 to 10 at all levels (reviewer-calculated). Mean-measured sediment concentrations were 7.7, 13, 24, 63, and 90 µg a.i./kg dw sediment, representing 60, 53, 49, 63, and 45% of nominal treatment levels, respectively. In pore water, reviewer-calculated percent changes were +900, +120, -21, -61, and -70% from Days 0 to 10 at the nominal 13, 25, 50, 100, and 200 µg a.i./kg sediment levels, respectively.

Nominal Sediment Conc. (µg a.i./kg)	Sediment, µg a.i./kg		Pore Water, µg a.i./L		Overlying Water	
	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10
Control	<0.94	<1.1	<0.00096	Not analyzed	Not assessed	Not assessed
S. Control	<0.94	<1.1	<0.00085	Not analyzed	Not assessed	Not assessed
13	9.0	6.5	0.010	0.10	Not assessed	Not assessed
25	16	11	0.015	0.033	Not assessed	Not assessed
50	28	21	0.080	0.063	Not assessed	Not assessed
100	80	46	0.16	0.062	Not assessed	Not assessed
200	140	41	0.37	0.11	Not assessed	Not assessed

B. Statistical Results

Statistical analyses were performed on amphipod survival and growth (dry weight). Analyses were performed using the response values for each replicate test vessel within a treatment level. Percent survival data were arcsine square-root transformed prior to analysis.

A t-Test was used to compare the performance of the negative control and solvent control data. Both endpoints were statistically similar, and the treatment groups were compared to the negative control data to determine treatment-level effects.

Normality of the data was evaluated using the Chi-Square Test, and homogeneity of variance was evaluated using Bartlett's Test. Survival data did not meet the assumption of homogeneity of variance and was therefore analyzed using the non-parametric Steel's Many-One Rank Test at the 95% level of certainty. Growth data met both assumptions and was thus analyzed using Bonferroni's Test at the 95% level of certainty. NOAEC and LOAEC values were assigned based upon significance.

Analyses were performed using TOXSTAT Version 3.5 statistical software and mean-measured sediment concentrations.

LC₅₀: 72 µg a.i./kg 95% C.I.: 57 to 78 µg a.i./kg
NOAEC: 63 µg a.i./kg
LOAEC: 90 µg a.i./kg

EC₅₀: 52 µg a.i./kg 95% C.I.: 47 to 58 µg a.i./kg
NOAEC: 24 µg a.i./kg
LOAEC: 63 µg a.i./kg

Statistical Method: The reviewer statistically analyzed data for day 10 survival and dry weight. For both endpoints the negative and solvent control data were compared using a Student's t-test and no significant difference was detected ($p>0.05$). The data for dry weight were further tested using Shapiro-Wilk's test to confirm normality and using Levene's test to confirm homogeneity of variances. The 90 $\mu\text{g a.i./kg}$ dry weight data were excluded from this analysis, due to a significant effect on survival at this level. Dry weight data satisfied the assumptions of ANOVA, so the NOAEC and LOAEC were determined using this test, followed by William's test (dose-dependent response). There was at least one group with zero variance for the survival data, so the NOAEC and LOAEC for this endpoint was determined using the non-parametric Steel's Many-One Rank test. These analyses were conducted using Toxstat 3.5 statistical software. The LC_{50} and EC_{50} values were determined using the moving average and Probit methods, respectively. For survival, the moving average method was selected over the Probit method because the Probit method could not provide a sound estimate (Goodness of fit probability = 0). The moving average method was run using Toxanal 2009 and the Probit method was run using Nuthatch statistical software.

$$\text{mg/kg OC} = \frac{\text{mg/kg dry weight}}{\text{kg TOC/kg dry weight}}$$

Based upon mean-measured sediment concentrations:

Survival:

LC₅₀: 60 µg a.i./kg 95% C.I.: 53 to 68 µg a.i./kg
Slope: N/A
NOAEC: 63 µg a.i./kg
LOAEC: 90 µg a.i./kg

Growth:

EC₅₀: 53 µg a.i./kg 95% C.I.: 42 to 65 µg a.i./kg
Slope: 3.54±0.78
NOAEC: 24 µg a.i./kg
LOAEC: 63 µg a.i./kg

Based upon ESTIMATED¹ pore water concentrations:

Survival:

LC₅₀: 0.007 µg a.i./L 95% C.I.: 0.006 to 0.007 µg a.i./L
NOAEC: 0.007 µg a.i./L
LOAEC: 0.01 µg a.i./L

Growth (dry weight):

IC₅₀: 0.006 µg a.i./L 95% C.I.: 0.005 to 0.007 µg a.i./L
Slope: 3.54±0.78
NOAEC: 0.003 µg a.i./L
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Based upon OC-normalized mean-measured sediment concentrations:

Survival:

LC₅₀: 938 µg a.i./kg TOC 95% C.I.: 828 to 1062 µg a.i./kg TOC
Slope: N/A
NOAEC: 984 µg a.i./kg TOC
LOAEC: 1406 µg a.i./kg TOC

Growth (dry weight):

EC₅₀: 828 µg a.i./kg TOC 95% C.I.: 656 to 1016 µg a.i./kg TOC
Slope: 3.54±0.78
NOAEC: 375 µg a.i./kg TOC
LOAEC: 984 µg a.i./kg TOC

13. REVIEWER'S COMMENTS:

The reviewer's NOAEC and LOAEC conclusions for survival and dry weight agreed with those of the study author; however, the LC₅₀ and EC₅₀ estimates slightly differed due to the different methods used to obtain these values. The reviewer's results were obtained using EFED-approved statistical programs, so they are reported in the Conclusions section.

Results were provided in terms of mean-measured sediment (bulk and OC-normalized) and estimated pore water concentrations in the Conclusions section of the DER.

Overlying water was not analyzed due to the pyrethroids' strong affinity to sediment (i.e., high K_{oc} values) and regular renewal of the overlying water. It was also reported that previous studies performed at the laboratory indicated that only negligible amounts of pyrethroids partition to overlying water (Springborn Smithers Laboratories Study Nos. 13656.6106, 13656.6107, 13656.6110, 13656.6111, and 13656.6112, Putt, 2005).

Analysis of the stock solution samples used to dose the test sediments ranged from 88 to 120% of nominal fortified concentrations. Pretest analysis of the spiked sediment following equilibration and prior to allocation into the replicate exposure vessels ranged from 27 to 63% of nominal concentrations. It was reported that while the results of the sediment pretest were variable, they were not thought to be indicative of test concentrations based upon sediment recoveries obtained at 0 and 10 Days.

During pore water analyses on Day 10, it was reported that due to a processing error, the control and solvent control solutions were inadvertently fortified with test substance; thus, results from these solutions were not reported/analyzed.

In pore water (measured at each level on Days 0 and 10), the redox potential was 150 to 270 mV on Day 0 and 2.9 to 8.6 mV on Day 10, the pH ranged from 6.8 to 7.1, the DOC ranged from 4.2 to 6.9 mg C/L, and the ammonia (as N) ranged from 3.8 to 9.7 mg/L.

This reviewer notes that the concentration of cypermethrin measured in pore water likely reflects both "freely dissolved" chemical (i.e., chemical that is not sorbed onto particulate organic carbon (POC) or dissolved organic carbon (DOC) in addition to dissolved chemical that is sorbed to DOC. This finding is indicated by the fact that the extraction and analytical methods used in this study do not distinguish among the two phases of chemical (freely dissolved and DOC-sorbed). It is also indicated by the much higher measured concentrations of cypermethrin in pore water than would be expected based on estimated values using sediment cypermethrin concentrations, its K_{oc}, and sediment total organic carbon (TOC). For highly hydrophobic chemicals like cypermethrin, DOC in pore water can substantially reduce its bioavailability and toxicity. It is further noted that the pore water estimated environmental concentrations (EECs) generated using the Agency's PRZM/EXAMS model are based on freely dissolved chemical. Therefore, some downward adjustment of these pore water toxicity values using appropriate methods (e.g., K_{oc} and DOC concentration in pore water) will likely be needed when comparing these values to

freely dissolved EECs generated using PRZM/EXAMS. Since the measured pore water concentrations of cypermethrin do not accurately describe the exposure to parent compound, endpoints from this study will not be expressed in terms of measured pore water concentrations.

Instead, this reviewer has estimated freely dissolved pore water endpoints based on measured concentrations in bulk sediment, the fraction of total organic carbon in bulk sediment (6.4%) and the mean K_{OC} (141,700 L/kg-OC, MRID 42129002) for cypermethrin. These estimated pore water endpoints, which are based on the freely dissolved test material (i.e., chemical that is not sorbed onto particulate organic carbon [POC] or dissolved organic carbon [DOC]), are consistent with the expression of aquatic estimated environmental concentrations (EECs) from PRZM/EXAMS. It is noted, however, that K_{OC} values for cypermethrin vary considerably depending on soil type (20,800 – 328,500 L/kg). This range of K_{OC} likely reflects differences in organic carbon composition and other soil properties used to determine K_{OC} . Therefore, these estimated pore water endpoints are subject to the same uncertainty in determination and application of K_{OC} for cypermethrin.

Nominal Sediment ($\mu\text{g a.i./kg}$)	Mean-measured Sediment ($\mu\text{g a.i./kg}$)	Estimated Pore Water ($\mu\text{g a.i./L}$)	OC-Normalized Sediment ($\mu\text{g a.i./g OC}$)
13	7.7	0.002	120
25	13	0.004	200
50	24	0.007	380
100	63	0.01	980
200	90	0.03	1400

A method validation report was not provided for California sediment 3. The analytical method used to quantify cypermethrin in California sediment 2 was validated in April 2009. Fortified samples were extracted two times with 50:50 methanol:purified reagent water and hexane; the extracts were combined and purified for analysis using solid phase extraction (SPE). Aliquots were analyzed using gas chromatography equipped with mass selective detection in negative chemical ionization mode (GC-MS/NCI). In samples fortified at 0.100 and 100 $\mu\text{g/kg}$, recoveries averaged $101 \pm 3.62\%$ and $88.7 \pm 1.63\%$, respectively, with a limit of quantitation (LOQ) of 0.0515 $\mu\text{g a.i./kg}$.

The analytical method used to quantify cypermethrin in freshwater was validated in January 2009. Fortified samples were acidified and extracted twice with ethyl acetate; the combined extracts were reduced in volume using rotary evaporation (30°C) and taken to dryness under nitrogen (room temperature). The residues were re-constituted in 0.1% peanut oil in acetone and analyzed using gas chromatography equipped with mass selective detection in negative chemical ionization mode (GC-MS/NCI). In samples fortified at 0.00100 (sample LOQ), 0.00300, 0.0200, and 0.0500 $\mu\text{g/L}$, recoveries averaged $114 \pm 3.82\%$. Due to the low concentrations being tested, the LOQ was set at 0.00100 $\mu\text{g/L}$; sample LOQ recoveries averaged $110 \pm 16.1\%$.

It was reported that representative samples of the overlying water source were periodically analyzed for pesticides, PCBs, and toxic metals, and that none of these compounds were detected

in any of the water samples analyzed in agreement with ASTM guidelines.

Definitive test dates were March 10 to 20, 2009.

This study was submitted to fulfill proposed OPPTS Draft 850.1735, whole sediment acute toxicity to freshwater invertebrates. This study is able to achieve a NOAEC for both the survival and growth endpoints.

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15. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Title: Day 10 % Survival

File: 6605s

Transform:

NO TRANSFORMATION

t-Test of Solvent and Blank Controls

Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean = 90.0000 Calculated t value = -1.9296

GRP2 (Blank cntl) Mean = 96.2500 Degrees of freedom = 14

Difference in means = -6.2500

2-sided t value (0.05,14) = 2.1448 No significant difference at alpha=0.05

2-sided t value (0.01,14) = 2.9768 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Day 10 % Survival

File: 6605s

Transform:

NO TRANSFORMATION

Steel's Many-One Rank Test

Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Neg Control	90.0000				
2	7.7	95.0000	80.00	46.00	8.00	
3	13	100.0000	92.00	46.00	8.00	
4	24	91.2500	71.00	46.00	8.00	
5	63	61.2500	48.00	46.00	8.00	
6	90	18.7500	36.00	46.00	8.00	*

Critical values are 1 tailed (k = 5)

NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE
OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY.
THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
90	80	65	81.25	0
63	80	31	38.75	0
24	80	7	8.75	0
13	80	0	0	0
7.7	80	4	5	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT
CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE
UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 68.92371

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	1.867002E-02	59.6691	53.21357	68.10691

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT
4	1.315806	13.70901	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.78465
 95 PERCENT CONFIDENCE LIMITS = -.4095824 AND 5.978883

INTERCEPT=-4.979327

LC50 = 61.3951
 95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC25 = 35.149
 95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 21.27631
 95 PERCENT CONFIDENCE LIMITS = 0 AND 47.68097

LC05 = 15.75546
 95 PERCENT CONFIDENCE LIMITS = 0 AND 37.02524

Title: Day 10 Dry Weight

File: 6605w Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean =	0.1463	Calculated t value =	-0.2483
GRP2 (Blank cntl) Mean =	0.1488	Degrees of freedom =	14
Difference in means =	-0.0025		

2-sided t value (0.05,14) = 2.1448 No significant difference at alpha=0.05
 2-sided t value (0.01,14) = 2.9768 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Day 10 Dry Weight

File: 6605w Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.0186
W = 0.9454

Critical W = 0.9170 (alpha = 0.01 , N = 39)
W = 0.9390 (alpha = 0.05 , N = 39)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Day 10 Dry Weight
File: 6605w Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	4	0.0004	0.0001	0.6329
Within (Error)	34	0.0057	0.0002	
Total	38	0.0061		

(p-value = 0.6425)

Critical F = 3.9273 (alpha = 0.01, df = 4,34)
= 2.6499 (alpha = 0.05, df = 4,34)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All equal (alpha = 0.01)

Title: Day 10 Dry Weight
File: 6605w Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	4	0.0510	0.0127	23.3550
Within (Error)	34	0.0186	0.0005	
Total	38	0.0696		

(p-value = 0.0000)

Critical F = 3.9273 (alpha = 0.01, df = 4,34)
= 2.6499 (alpha = 0.05, df = 4,34)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Day 10 Dry Weight

File: 6605w

Transform:

NO TRANSFORMATION

Bonferroni t-Test

- TABLE 1 OF 2

Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	t STAT	SIG
0.05					
1	Neg Control	0.1463	0.1463		
2	7.7	0.1400	0.1400	0.5350	
3	13	0.1450	0.1450	0.1070	
4	24	0.1388	0.1388	0.6420	
5	63	0.0486	0.0486	8.0777	*

Bonferroni t critical value = 2.3451 (1 Tailed, alpha = 0.05, df = 4,34)

Title: Day 10 Dry Weight

File: 6605w

Transform:

NO TRANSFORMATION

Bonferroni t-Test

- TABLE 2 OF 2

Ho: Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	8			
2	7.7	8	0.0274	18.7	0.0063
3	13	8	0.0274	18.7	0.0013
4	24	8	0.0274	18.7	0.0075
5	63	7	0.0284	19.4	0.0977

Title: Day 10 Dry Weight

File: 6605w

Transform:

NO TRANSFORMATION

William's Test

- TABLE 1 OF 2

Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN

1	Neg Control	8	0.1463	0.1463	0.1463
2	7.7	8	0.1400	0.1400	0.1425
3	13	8	0.1450	0.1450	0.1425
4	24	8	0.1388	0.1388	0.1388
5	63	7	0.0486	0.0486	0.0486

Title: Day 10 Dry Weight

File: 6605w

Transform:

NO TRANSFORMATION

William's Test - TABLE 2 OF 2

Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	0.1463				
7.7	0.1425	0.3210		1.7000	k= 1, v=30
13	0.1425	0.3210		1.7800	k= 2, v=30
24	0.1388	0.6420		1.8000	k= 3, v=30
63	0.0486	8.0777	*	1.8100	k= 4, v=30

s = 0.0234

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower Upper	Std.Err.	Lower Bound /Estimate
EC5	18.	9.4 35.	0.14	0.52
EC10	23.	13. 40.	0.12	0.58
EC25	34.	23. 50.	0.083	0.68
EC50	53.	42. 65.	0.046	0.81

Slope = 3.54 Std.Err. = 0.780

Goodness of fit: p = 0.43 based on DF= 3.0 39.

6605WE : Day 10 Dry Weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	8.00	0.146	0.146	-0.000144	100.	0.00
7.70	8.00	0.140	0.146	-0.00617	99.8	0.155

13.0	8.00	0.145	0.144	0.000913	98.4	1.58
24.0	8.00	0.139	0.130	0.00901	88.6	11.4
63.0	7.00	0.0486	0.0571	-0.00855	39.0	61.0
90.0	6.00	0.0350	0.0298	0.00516	20.4	79.6